Monitoring Adherence with Biofilms

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Why look at biofilms?

• A second method for measuring adherence that is independent of self-reporting

• The perfect method would be able to tell us how long the ring was worn

• There is some preliminary data suggesting the quantity of biofilm may correlate to time VR are worn
What are Biofilms

- Aggregates of bacteria within a matrix
- The bacterial secrete biopolymers to make the matrix, also called extracellular polymeric substrate (EPS)
  - Carbohydrates, proteins, extracellular DNA, lipids
- The matrix is responsible for adhesion to surfaces
- The biofilm become more complex over time
Detection of Biofilms with Scanning Electron Microscopy

Unused ring

Ring worn for 28 days
Bacteria with biofilm
Detecting EPS with FISH

*Gardnerella vaginal* stained with DAPI and WGA

- **Blue**: nucleus of cells
- **Pink**: EPS
Quantifying Biofilm

- Study of Aciclovir delivery via vaginal ring for reduction of HSV transmission
- 6 women with recurrent genital HSV
  - 3 wore ring for 7 day, 3 women 14 days
- Comparison of the two groups showed the IVR worn for 14 days showed a more complex biofilm covering a larger portion of the ring than those worn for only 7 days. (Keller, MJ. J of Antimicrob. Chemotherapy 2012)
Processing Rings to Ensure Accurate Detection

• When removing the ring from the vagina limit the amount of twisting and handling
• Don’t wipe the ring
• Immediately place ring in container with 2.5% paraformaldehyde
• Must be fixed for 1 to 8 hours before the laboratory will prepare for storage and shipment
• If participant brings the ring into the clinic in a bag transfer it to the container with paraformaldehyde
Discoloration of ring
Ring is stained on the outside only
Any questions or comments?
Immediately place ring in container with 2.5% paraformaldehyde after removing from vagina.

Cap tightly and transport the day of collection to the lab for processing and storage.